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Triazole derivatives: A series of Darapladib analogues as orally active Lp-PLA$_2$ inhibitors

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ABSTRACT

This Letter reports our efforts towards the optimization of our previously identified series of imidazole and triazole derivatives that lead to the discovery of a series of orally active Lp-PLA$_2$ inhibitors in C57 mice. These inhibitors are characterized by the presence of a diamine side chain in the molecules, such as **2c**, **2f**, and **4a**. The introduction of the terminal-end amine succeeded in maintaining the in vitro activities at sub-nanomolar levels. The vivo activities could be greatly affected by variations in the two amines via modulating the metabolic stability and lipophilicity of the compounds.

Clinical studies have convincingly shown that a substantial residual risk persists in cardiovascular patients despite aggressive treatment with a variety of different therapeutic agents in combination, including lipid lowering drugs, hypotensors and antiplatelets. The development of new drugs with novel pharmacological mechanisms to complement the existing pipelines and treat the residual cardiovascular risks are therefore urgently required.[1] In recent years, a deeper understanding of the role of inflammation in the pathogenesis of atherosclerosis has been developed and resulted in the identification of a significant number of novel inflammatory mediators.[2] Of these new mediators, lipoprotein-associated phospholipase A$_2$ (Lp-PLA$_2$) has been identified as a crucial enzyme in atherosclerosis, and is generally believed to catalyze the hydrolysis of oxidatively modified phospholipids at the sn-2 position within low density lipoproteins (LDLs).[3] The hydrolysis products, including non-esterified fatty acids (NEFA) and lysophosphatidylcholine (LysoPC), are well-known pro-inflammatory factors that are involved in every stage of the atherogenesis process.[4] The accumulation of evidence from both animal and epidemiologic studies has demonstrated that Lp-PLA$_2$ is an independent predictor of cardiovascular risk.[5] Accordingly, Lp-PLA$_2$ has come to be regarded as a promising new target for atherosclerosis, with this understanding being boosted by the phase III development of Darapladib,[6] which is a pyrimidone Lp-PLA$_2$ inhibitor developed by GSK scientists.

In addition to this pyrimidone class of Lp-PLA$_2$ inhibitors and its analogues, several other Lp-PLA$_2$ inhibitors have also been disclosed, including carbonyloxime,[7] amides of xanthurenic acid,[8] and, most recently, a series of carbamates.[9] None of these three classes, however, has been reported to be efficacious in vivo. To enrich the family of Lp-PLA$_2$ inhibitors, we have attempted to explore a series of Darapladib analogues by replacing the amide group of Darapladib with an imidazole or a triazole ring (Fig. 1).[10] The resulting compounds, exemplified by compounds **1a** and **1b** (Table 1), exhibited unsatisfactory levels of in vitro activity. In-house structure–activity relationship (SAR) studies revealed that modifications to the 4-triflurobiphenyl and 4-flurobenzyl moieties had little effect on potency enhancement. Consequently, we attempted

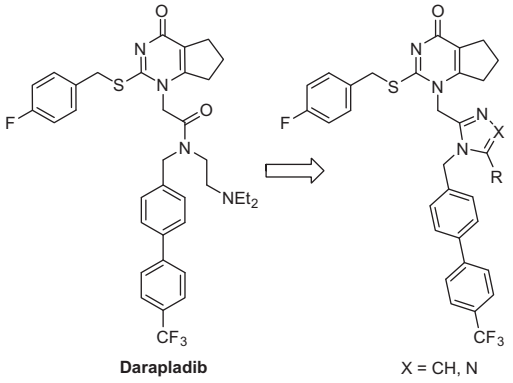

Figure 1. Replacement of the amide of Darapladib with an imidazole or a triazole.

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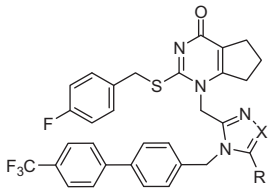
Table 1
Inhibitory activities of the synthesized compounds in rabbit and human plasma assays, as well as the IC_{50} values of selected compounds in recombinant human Lp-PLA_2 assay



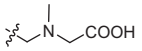
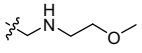
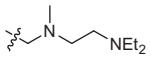
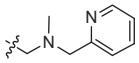
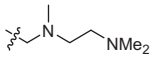
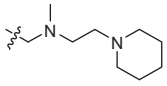
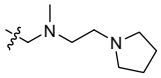
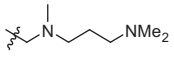
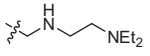
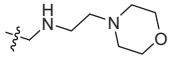
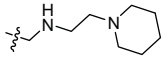
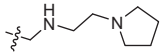
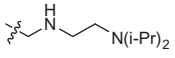
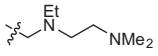
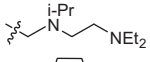
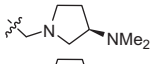
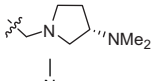
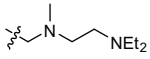
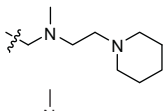
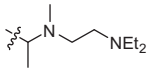
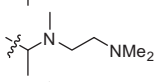
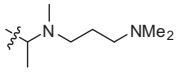
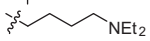
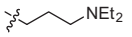
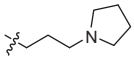
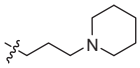
Compound[c]	X	-*R*	% Inhibition in rabbit plasma		% Inhibition in human plasma	rhLp-PLA_2 IC_{50} (nM)
			100 nM	10 nM	10 nM	
1a	CH		88	10	75	NT[a]
1b	N		85	21	78	NT
2a	N		74	5	76	NT
2b	N		75	13	76	NT
2c	N		98	72	94	0.91
2d	N		91	36	91	NT
2e	N		97	77	95	0.14
2f	N		97	75	94	0.67
2g	N		97	76	92	0.23
2h	N		98	73	93	0.55
2i	N		97	35	82	NT
2j	N		98	32	85	NT
2k	N		97	33	79	NT
2l	N		98	55	82	NT
2m	N		97	41	82	NT
2n	N		97	75	92	NT
2o	N		95	33	81	NT
2p	N		98	54	89	1.60
2q	N		99	46	86	NT
3a	CH		89	54	81	1.72
3b	CH		92	54	84	NT
4a	N		96	76	92	0.24
4b	N		96	71	94	0.81
4c	N		96	71	93	0.89
5a	N		95	74	93	0.46

Table 1 (*continued*)

Compound[c]	X	-*R*	% Inhibition in rabbit plasma		% Inhibition in human plasma	rhLp-PLA$_2$ IC$_{50}$ (nM)
			100 nM	10 nM	10 nM	
5b	N		96	73	91	0.96
5c	N		97	75	93	0.86
5d	N		96	76	92	0.95
Darapladib			99	82	95	0.10[b]

[a] Not tested.
[b] Reported IC$_{50}$ = 0.25 nM.
[c] Compounds **1a** and **b** are published compounds in Ref. 10. Compounds **2a–5d** are new compounds.

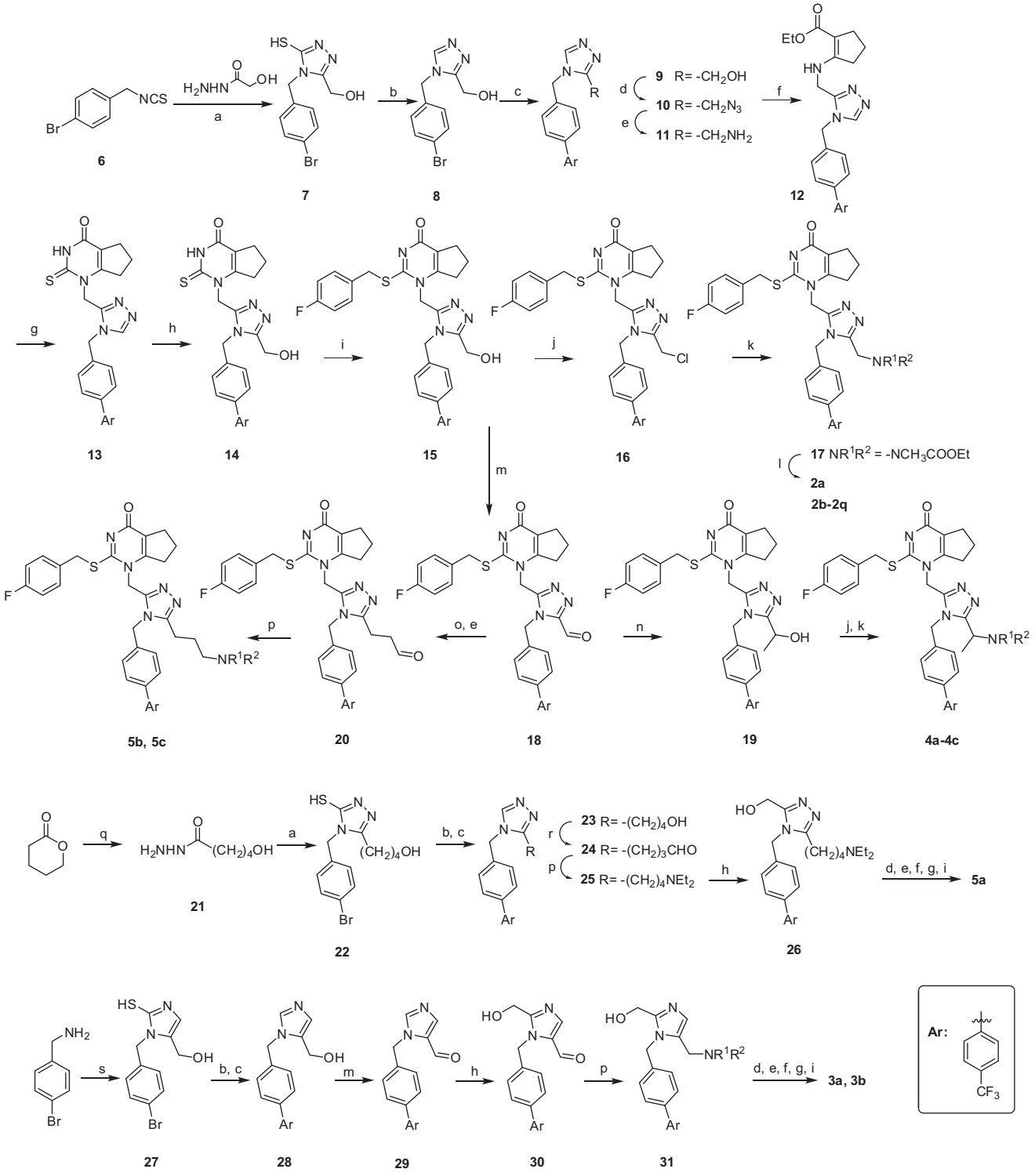
to increase the potency by making further modifications of the R region, namely the side chain of the molecule. Herein, we describe our recent work towards the optimization of the side chain that led to the identification of orally active Lp-PLA$_2$ inhibitors in C57 mice.

Compounds **2a**–**q** were prepared according to a slightly modified version of our previously published procedure,[10] which started with the condensation reaction of 1-bromo-4-isothiocyanoatomethylbenzene with 2-hydroxyacetohydrazide under basic condition, to afford intermediate **7** (Scheme 1). Subsequent oxidative desulfurization followed by Suzuki coupling gave alcohol **9**, which was converted to the corresponding azide **10** in the presence of diphenylphosphoryl azide (DPPA). The azide **10** was then converted to amine **11** using catalytic hydrogenation condition. The enamine reaction of **11** with ethyl 2-oxocyclopentanecarboxylate provided intermediate **12**, followed by a ring closing reaction in the presence of trimethylsilyl isothiocyanate to give thiouracil **13**, which was subsequently heated in a formaldehyde solution at reflux to yield **14**. The alkylation of **14** with 4-fluorobenzyl bromide provided the key intermediate **15**. Treatment of this intermediate with $SOCl_2$ provided the corresponding chloride **16**, which was reacted with a variety of different amines to give the desired target compounds **2a**–**q**. Intermediate **15** was also oxidized to aldehyde **18** which was subsequently treated with CH_3MgBr to afford **19**, followed by chlorination and substitution reactions to afford compounds **4a**–**c**. The formaldehyde **18** was converted to propylaldehyde **20** via a two steps procedure involving Wittig reaction and catalytic hydrogenation. The aldehyde **20** was then converted to compounds **5b** and **5c** using reductive amination chemistry. The synthesis of **5a** began with the hydrazinolysis of δ-valerolactone to give **21**, which was subjected to the same procedures used for the preparation of **9** to give intermediate **23**. The application of successive Swern oxidation, reductive amination and hydroxymethylation reactions provided alcohol **26**, which was subsequently converted to **5a** according to the routine procedures described above. The preparation of the imidazole derivatives **3a** and **3b** was performed in a similar manner to that of **5a**, except for the construction of intermediate **27**[11] and the oxidation of alcohol **28**.

The inhibitory activities of the compounds against the enzyme of Lp-PLA$_2$ were evaluated in rabbit and human plasmas according to the reference method[12] and expressed as the percentage inhibition of the enzyme activity (Table 1). The activities observed in plasma factored in non-specific binding effects and were more instructive for the SAR studies. The most promising compounds were also evaluated their IC$_{50}$ values using recombinant human Lp-PLA$_2$ (rhLp-PLA$_2$).

Our optimization approach involved the introduction of an O- or N-containing functional group into the side chain with the expectation that the group would form meaningful hydrogen-bonding or electrostatic interactions with the protein. Only a few analogues (**2a**–**2d**) were prepared initially to quickly evaluate the potential of these compounds. Pleasingly, this limited panel of compounds provided some exciting results. Based on the data shown in Table 1, it was clear that the introduction of O-containing groups provided no increase in activity (**2a**, **2b**). In contrast, the introduction of N-containing groups was greatly favored, with compound **2c** displaying a level of activity comparable to that of Darapladib in rabbit and human plasma assays. The pyridine-containing compound **2d** was less potent than the diethylamine derivative **2c**, suggesting that an sp^3-N was more efficacious than an sp^2-N. Consequently, the type of ethylenediamine side chain became the focus of our study and several different analogues were synthesized containing variations to the terminal amine. As expected, the frequently used amines (**2e**–**g**) displayed similar levels of inhibitory activity in rabbit and human plasma assays and increased potency against rhLp-PLA$_2$ in comparison with **2c**. Furthermore, a one-carbon atom extension between the two nitrogen atoms afforded compound **2h**, which also possessed good binding affinity. In general, the introduction of the amine to the terminal-end of the molecule has led to significant improvements in the inhibitory activity, likely due to the occurrence of an electrostatic interaction between the protonated nitrogen atom and the protein. The introduction of the amine was also favored in the imidazole series, although the resulting compounds (**3a**, **3b**) were less potent than the corresponding triazoles. With this in mind, the decision was taken to continue the optimization work primarily with the triazole series.

We then proceeded to explore the nature of the amine at the internal-end of the side chain via the incorporation of a variety of different substituents. In comparison with the methyl-substituted derivatives, the corresponding un-substituted secondary amines showed reduced levels of inhibition towards the enzyme (**2i**–**m**). Although the replacement of the methyl group with an ethyl group was well tolerated (**2n**), replacement with an isopropyl group significantly weakened the inhibitory activity (**2o**). The inclusion of the N atom within a ring system led to a significant loss in the activity (**2p**, **2q**), demonstrating the importance of the flexibility of the side chain to the high binding affinity. The results from previous study[10] implied a possible preference for the introduction of further steric bulk alpha to the heterocycle. With this in mind, several compounds were prepared bearing a branched methyl group to determine its effect on the potency (**4a**–**c**). Unfortunately, however, the additional methyl group did not provide any further enhancement to the inhibitory activities of the compounds. Finally, we assessed the potential of replacing the N atom with an sp^3-C atom. The resulting butylamine derivative **5a** provided a similar level of inhibition to that of the diamine analogue **2c**, indicating that the internal-end N of the side chain was not important to the observed in vitro activity and could therefore be replaced without loss in potency.



Scheme 1. Reagents and conditions: (a) EtOH, reflux, 2 h, then K_2CO_3, H_2O, reflux, 1 h; (b) H_2O_2 (30 wt % sol. in water), AcOH, CH_2Cl_2, reflux, 1 h; (c) 4-trifluoromethylphenylboronic acid, Cs_2CO_3, $Pd(Ph_3P)_4$, dioxane, reflux, 18 h; (d) DPPA, DBU, THF, reflux, 3 h; (e) H_2, 10%Pd/C, 1 atm, EtOH, rt, 12 h; (f) ethyl 2-oxocyclopentanecarboxylate, $Si(OEt)_4$, EtOH, reflux, 3–5 h; (g) $(CH_3)_3SiNCS$, DMF, 140 °C, 4 h; (h) formaldehyde (37 wt % sol. in water), reflux, 8 h for **14**, **25** or 36 h for **29**; (i) 4-fluorobenzyl bromide, DBU, KI (cat.), CH_3CN, rt, 5 h; (j) $SOCl_2$, CH_2Cl_2, DMF (cat.), 0 °C, 1 h then $NaHCO_3$ solution; (k) HNR^1R^2, DBU, KI (cat.), CH_3CN, reflux, 1 h; (l) NaOH, H_2O–EtOH, rt, 2 h, then 6 N HCl; (m) MnO_2, dioxane, 70 °C, 3 h; (n) CH_3MgBr, THF, 0 °C, 2 h; (o) Ph_3P = CHCHO, THF, rt, 12 h; (p) HNR^1R^2, $NaBH(OAc)_3$, CH_2Cl_2, rt, 2 h; (q) H_2NNH_2–H_2O, EtOH, reflux, 2 h; (r) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −60 °C; (s) concd HCl, 0 °C, CH_3CN, then 1,3-dihydroxyacetone, propionic acid, KSCN, 70 °C, 2 h.

Furthermore, the analogues containing one carbon less in their side chains (i.e., the propylamine derivatives **5b**–**d**) exhibited a similar level of inhibition to that of **5a** in rabbit and human plasma assays. Together with the data from compounds **2h** and **4c**, these results reveal that side chains consisting of three to five atom-tethered amines all can be well accommodated by the active site of the enzyme in this particular region.

Based on the in vitro SAR studies, the most promising compounds were subjected to preliminary evaluation in our C57 mouse assay. The testing compounds were formulated in tartrate salts for

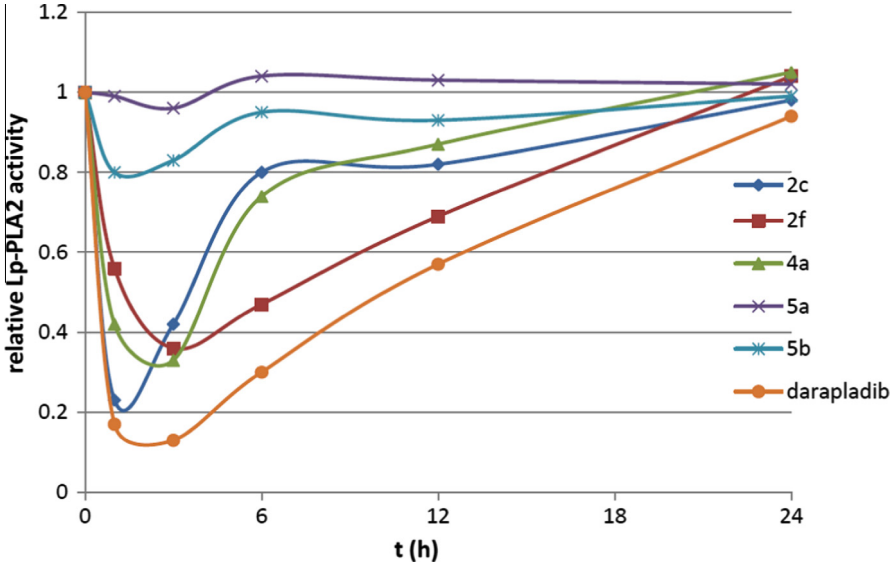


Figure 2. Relative serum Lp-PLA_2 activities in C57 mice after a single dose (50 mg/kg, po, $n = 5$).

oral administration at 50 mg/kg.[13] As depicted in Figure 2, the piperidine derivative **2f** displayed the most prolonged level of inhibition with activity extending over a 12-h period. The diethylamine analogues **2c** and **4a** showed enhanced levels of inhibition whereas the efficacy lasted over a shorter period of time compared with **2f**, suggesting that cyclic amine might possess better metabolic stability than acyclic one. In contrast, the propylamine derivative **5b** was found to be a much weaker inhibitor in vivo, whereas the butylamine derivative **5a** was nearly inefficacious. The attenuation of in vivo activities might be attributed to the poor absorption properties of **5a** and **5b** which were caused by the significantly elevated log*P* values as a consequence of removing the internal-end N atom (8.56, 9.36 and 8.94 for **2c**, **5a** and **b**, respectively, calculated using ChemBioDraw Ultra 11.0). It has been shown in the SAR studies that high binding affinities were well regulated by the presence of the terminal-end amine. Herein, the studies in mice revealed that variations in the two amines were able to change the metabolic stability and lipophilicity of the analogues, leading to quite different in vivo profiles. These findings indicated the possibility of further improving the in vivo performances of our compounds by rational modifications of the side chain, although they are still incomparable to Darapladib at present.

In summary, we have designed and synthesized a series of orally bioactive Lp-PLA_2 inhibitors in C57 mice by making precise modifications to the side chain of our previously discovered triazole scaffold. The most promising compounds, including compound **2c**, **2f**, and **4a** featured a diamine side chain, in which the terminal-end amine provided binding interaction with the protein as the key factor for high affinity. The analogues that were similarly potent in vitro gave very different in vivo performances, probably due to the differences in their metabolic or absorption properties that could be modulated by the two amines. The research findings disclosed in this study should prove of great value in further design of improved Lp-PLA_2 inhibitors.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.03.062.

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12. Activities in rabbit, human plasma and recombinant human Lp-PLA_2 (rhLp-PLA_2) assays were all assessed using [^{3}H]PAF as the substrate. Briefly, DMSO solutions of the compounds were added to assay buffer containing 50 mmol/L HEPES (pH 7.4), 150 mmol/L NaCl, 150 μL dd H_2O and 10 μL plasma (or rhLp-PLA2) in a total volume of 200 μL. Following 5 min of preincubation at 37 °C, 10 μL [^{3}H]PAF (1uCi) was added and incubated for 10 min at 37 °C. After the incubation, 600 μL of $CHCl_3$/MeOH (2:1) was added and the resulting mixture was mixed thoroughly. After a period of centrifugation at 12000*g* for 15 min at 4 °C, 200 μL of the supernatant was collected and mixed with 200 μL of $CHCl_3$. An 80 μL portion of the supernatant was then collected and its radioactivity was measured in a liquid scintillation counter. The inhibition rate was determined by the following equation:Inhibitory rate (%) = $1 - (CPM_{compound} - CPM_{blank})/(CPM_{positive} - CPM_{blank})^*100\%$The blank sample contained no plasma or test compound in the assay buffer and the positive sample contained no test compound.
13. A group of five C57 mice that had been fasted for 16 h were administrated with the test compound (50 mg/kg po) and blood samples were drawn before or 1, 3, 6, 12, and 24 h after administration to measure the serum Lp-PLA_2 activity according to the method described in Ref. 10.